EFFECTS OF CADMIUM ON WHITE-TAILED PTARMIGAN IN COLORADO

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ABSTRACT

Cadmium is known to be toxic to vertebrates when test organisms are exposed to sufficiently high dietary concentrations. But because cadmium occurs as a trace constituent of most ecosystems, it has rarely been observed to have toxic effects on natural populations of wildlife. This paper summarizes the results of a multi-disciplinary study of the effects of metals on wildlife living in the ore-belt region of Colorado. It reports that white-tailed ptarmigan (*Lagopus leucurus*) in this region are: 1) exposed to uncharacteristically high levels of cadmium through their diets; 2) accumulate potentially toxic cadmium concentrations in their kidneys after just 700 days of exposure, and that approximately half of adult ptarmigan in the region; 3) experience cadmium-induced nephrosis of kidney tissue and, probably as a result; 4) develop calcium-poor leg bones. Additionally, this paper suggests that ptarmigan may not be the only herbivores in the region to be affected by cadmium but rather, may be indicators of a broader problem affecting ecosystems generally in central and southwestern Colorado.

INTRODUCTION

C.E. Braun of the Colorado Division of Wildlife (CDoW) reported high mortality rates and low reproductive success among certain populations of White-tailed Ptarmigan in Colorado (Braun 1969:62). At the time, Braun attributed these differences in fitness to some unknown environmental factor, possibly "quality of habitat." Later, while working in the Animas River watershed in the San Juan Mountains of southwestern Colorado, Braun captured ptarmigan with unusually brittle bones (pers. comm.).

Over the past six years, I have studied the effects of trace metals on White-tailed Ptarmigan in the Animas River watershed in southwestern Colorado (Larison et al. 2000; Larison et al. 2001; Crock et al. 2000; Larison 1999, 2001;) attempting to determine if the Braun observations were somehow linked to one or more of the metals common to central and southwestern Colorado. Little is known about the effects of metals on natural populations. Most trace metal research to date has focused on "acute" rather than "chronic" exposure and has used captive animals, high dose rates, and short exposure times. Because the effects of a single, large dose are often quite different from those produced by repeated, small doses (Eaton and Klaasen 1996), studies of acute toxicity are only marginally useful in assessing risk to natural populations. In addition, most studies of metals toxicity among natural populations have been done in aquatic ecosystems. Few investigators have explored the effects of chronic metals exposure on terrestrial organisms or populations.

That we know so little about the effects of metals on natural populations is particularly troubling because human activities tend to mobilize metals (Lantzy and MacKenzie 1979, Nriagu 1979, Nriagu 1980, Nriagu and Pacyna 1988). In particular, mining activities such as those common to Colorado have contributed to a worldwide build-up of metals in biologically sensitive places (Roberts and Johnson 1978). Rising levels of contamination have been detected in even some very remote ecosystems having been transported there by wind (Nriagu 1979). Nriagu and Pacyna (1988:139) have said, “mankind has become the most important element in the global biogeochemical cycling of the trace metals.” Concern exists that anthropogenic mobilization of trace metals is expanding the geographic boundaries of the metals problem and increasing the numbers of organisms and species affected.

A number of investigators have shown that these metals can have a cumulative effect on captive test subjects (Friberg et al. 1986); once herbivores ingest them, these metals begin to build up in kidney and liver tissues. When dietary exposure levels are high enough or when exposure times are long enough, some trace metals will eventually reach toxic levels in the kidney (Friberg 1952, Nordberg 1978). When test subjects are exposed to sufficient concentrations of metals, renal tubules eventually fail, producing a condition of metals stress (Richardson et al. 1973, Webb 1979, Elinder et al. 1981, Nicholson and Osborn 1983). Because damaged kidney tubules are less efficient than undamaged tubules, it has been suggested that a damaged kidney would be unable to maintain appropriate serum electrolyte balances and would permit calcium to be excreted (Ceresa 1945, Hiroto 1971, Hook and Hewitt 1986, Kido et al. 1988). In a cascading effect, calcium would be lost from bone tissue as the body borrows from the skeleton to make up for serum losses (Chang et al. 1980, Bhattacharyya 1991, Sacco-Gibson 1992). A number of researchers have documented a causal relationship between one such metal—cadmium—and skeletal weakening in test animals (Ceresa 1945, Larsson and Piscator 1971, Bhattacharyya 1991). This relationship has been reviewed thoroughly by Cooke and Johnson (1996) and Furness (1996).

METHODS

Over a period of six years, I used a multidisciplinary approach to overcome some inherent difficulties associated with the study of environmental toxins, *in situ*. I combined geological, botanical, physiological, and demographic studies to answer questions about possible metals poisoning in the White-tailed Ptarmigan, an herbivore (Larison et al. 2000, Larison et al 2001, Larison 2001). I began with the broad hypothesis that metals were responsible for the brittle-bone condition observed by Braun among certain populations of White-tailed Ptarmigan in southwest Colorado. I postulated further that metals stress might have reduced fitness in at least some of these populations. To better understand how metals stress might be linked to the brittle-bone condition and to fitness, I generated a model and then collected new data to test assumptions about possible mechanisms involved. I asked: i) what potentially toxic trace metals are present in the study area; ii) how specifically are ptarmigan exposed to these metals, if they are exposed; iii) what tissues are affected by these metals; and iv) what life history changes occur in association with these metals. In the process, I traced metals through the ptarmigan diet, monitored accumulation rates and effects in target organs, examined tissue responses and damage, and evaluated possible effects on reproductive success and survival.

Ptarmigan Foods

Using ICP-AES total analysis, after Crock et al. (1999), I evaluated all plant species known to be consumed by ptarmigan for metals (i.e. Cd, Cu, Zn, Pb, Fe) and salts (Larison 2001). Samples of six different plant genera were collected from four study sites. These sites were scattered along the mineral zone of Colorado from Guanella Pass near Mt. Evans (39º 33'

N; 105º 42' W) to the San Juan Mountains (37º 54' N; 107º 44' W) north of Durango. Several of these sites are near abandoned mine sites; others were relatively metals free.

I sampled leaf buds and apex stems of several species of willow (*Salix spp*.) in winter. In summer, both erect and prostrate varieties of willow were sampled, as were *Trifolium* spp., *Geum rossii, Bistorta bistorta, Carex ebenea,* and *Dryas octopetala*. Samples were taken only where birds were observed to feed. Each feeding area was sampled comprehensively. Each sample consisted of at least 200 g of plant tissue from as few as 10 or from as many as 300 independent plants, depending on species. Samples were collected either with powderless gloves or with stainless-steel scissors. Only those parts of each plant that were known to be preferred ptarmigan foods (Quick 1947, Braun 1969, Braun et al. 1993) were collected.

The physical preparation of materials were done by chemists at the USGS laboratory in Denver and consisted of drying, milling, and ashing after Peacock (1992). Samples were not washed because ptarmigan eat unwashed plant matter. Ashing was done in a muffle furnace, programmed to slowly ramp up to 500° C over a 5-hour period. Complete ashing was assured by maintaining this temperature for at least 12 hours. The furnace subsequently was allowed to cool for 8 hours before samples were removed. Samples were digested in a cocktail of acids (including hydrofluoric, hydrochloric, nitric, and perchloric) at low temperature and pressure, after Crock et al. (1983). Crock et al. (1999) report that cadmium and zinc both are digested completely by this procedure.

Animal Tissue

Using multi-metal tissue analysis, I measured cadmium concentrations in two geographically distinct populations to determine whether elevated metals concentrations existed in liver and/or kidney tissue of White-tailed Ptarmigan inhabiting the minerals-rich zone of Colorado (Larison et al. 2000, Larison 2001). The study population inhabited the mineral zone west and southwest of Denver, Colorado. The reference population inhabited Indian Creek and Ahtell Creek north and northeast of Anchorage, Alaska.

Multi-metal total analyses of tissues were performed by means of inductively coupled plasma-atomic emission spectroscopy (ICP-AES) by chemists in two independent laboratories, after Crock et al. (1999). At each laboratory, 1.0 g samples of tissue were digested in ultra-pure concentrated nitric and hydrochloric acids. The resultant ash was brought to a total volume of 10 ml in a matrix-matched nitric acid dilution. Multi-metal scans were made of each sample. At the UC-Davis Veterinary Diagnostic Laboratory, NIST bovine liver (1577b) and NRCC TORT-2 standards were run, as was a method blank and Cd-spiked samples. The TORT-2 was assayed at 26.7 ppm Cd, 106 ppm Cu, and 180 ppm Zn. At the Cornell University ICP Laboratory, a bovine NBS standard (1577b), assayed at 0.5 ppm Cd, 160 ppm Cu, and 127 ppm Zn was used.

Histopathologies were done on 39 kidneys and 12 pancreases at the UC-Davis Veterinary Diagnostics Laboratory. These tissues were embedded in wax, stained, thin-sectioned, and examined at varying magnifications using a light microscope. Tissues were examined for evidence of tubular damage and/or failure.

Bone Tissue

Bone tissue was analyzed for the presence of metals and for the salts concentrations (Larison 2001). Lyophilized samples were crushed, placed in acid-washed crucibles, and covered with 20 % trace-metals-grade nitric acid for 2 hours. Samples were placed in a muffle furnace, “ramped up” to 450° C and held at this temperature overnight. Ashed samples (0.2 g) were digested in a cocktail of 2 ml concentrated nitric, 1 ml concentrated hydrochloric, and 2 ml concentrated hydrofluoric acids, after Briggs and Meier (1999). A 1:10 dilution of the sample was made using 1 % nitric acid. J. Crock, a USGS geochemist, analyzed these samples using an Inductively Coupled Mass Spectrometer (ICP-MS–Perkins Elmer Elan 6000). A dual detector calibration and auto-lens adjustment was performed prior to machine use, following manufacturer’s specifications. Two calibration standards and a 1 % nitric acid blank were run with each batch of samples.

RESULTS

Plant Tissue

Noteworthy levels of cadmium, zinc, and copper were detected in all ptarmigan food resources tested (Table 1). Cadmium concentrations ranged from 0.1 to 11 µg g $^{-1}$. Zinc levels ranged from 29 to 619 µg g $^{-1}$. Copper concentrations ranged from 1 to 21 µg g $^{-1}$. Metals concentrations were highest in plants belonging to the genus *Salix*. In the willows, cadmium levels were found at concentrations significantly above those reported elsewhere in North America (Peterson and Alloway 1979, Page et al. 1980).

Table 1: Mean metals concentrations in six plant genera collected in the mineral zone of Colorado (mg k $^{-1}$ – dw).

Plant Genus	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Geum	0.23	0.29	1.62	6.81	2000	90	2.36	0.25	30.2
Bistorta	0.14	0.38	1.27	7.22	2000	238	3.61	0.68	53.2
Carex	0.15	0.60	2.20	7.60	3000	483	6.00	0.30	48.0
Dryas	0.20	0.30	3.40	5.00	7000	135	2.21	3.10	29.0
Trifolium	0.23	0.58	1.21	6.11	2000	139	2.10	0.44	51.5
Salix	2.84	0.84	1.45	6.46	2000	312	2.95	1.02	187

Animal Tissue

Elevated renal cadmium, zinc, and copper concentrations and elevated hepatic-cadmium concentrations were detected in White-tailed Ptarmigan throughout the mineral zone of Colorado (Figure 1). Among nine adult ptarmigan collected on and near Mt. Evans and Guanella Pass, four (44%) had kidney-Cd levels greater than 100 µg g $^{-1}$; all had levels greater than 50 µg g $^{-1}$. Among 25 adult ptarmigan collected in the Animas River watershed, 12 (48%) had kidney-Cd levels greater than 100 µg g $^{-1}$; all had levels greater than 40 µg g $^{-1}$. In contrast, birds in the reference population had only moderately elevated kidney-Cd levels as compared to the norm for birds. Only a single bird collected outside the mineral zone had a kidney-Cd level greater than 40 µg g $^{-1}$. In the mineral zone, 27 % of ptarmigan had kidney-Cd levels greater than 100 µg g $^{-1}$, 94 % had elevated levels greater than 20 µg g $^{-1}$, and only 6 % (all chicks) had kidney-Cd levels below 20 µg g^{-1}. Outside the mineral zone, 96 % of birds had kidney-Cd levels below 20 µg g^{-1}. Only a single Alaskan White-tailed Ptarmigan had an elevated kidney-Cd concentration. The differences in means between populations (Colorado mineral zone versus non-mineral zone) were highly significant for kidney-cadmium, zinc, and copper as well as for hepatic-cadmium ($P < 0.001$ in all four cases) and not significant for liver-copper or for liver-zinc ($P > 0.5$).

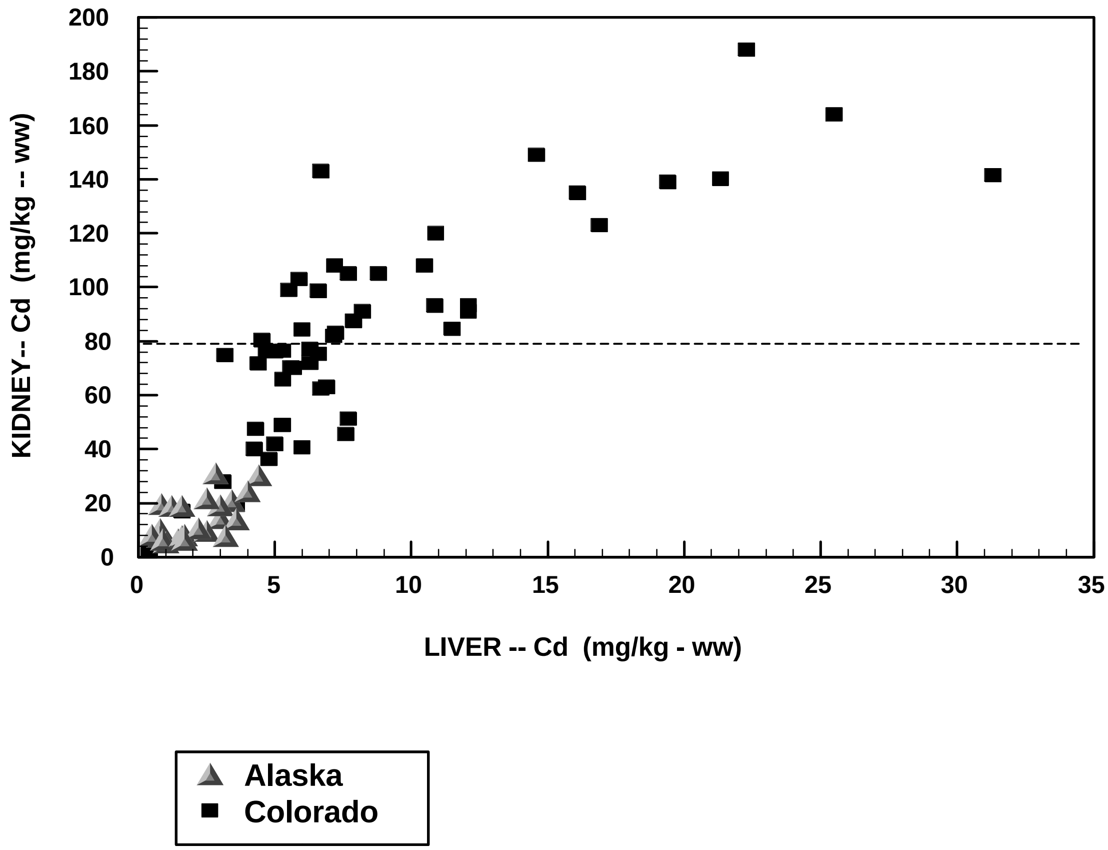

Figure 1. Kidney- and liver-cadmium levels in two populations of White-tailed Ptarmigan. Difference in means between populations is highly significant ($P<0.0001$). Toxic threshold (dashed line) from Furness 1996.

Kidney-Cd and liver-Cd concentrations were correlated. A nearly linear relationship appeared at cadmium concentrations below the toxic threshold, but a reduction in the rate of cadmium accumulation occurred at higher kidney-Cd values.

Kidney-Cd levels were markedly age-dependent among ptarmigan in Colorado (Table 2). Chicks were relatively cadmium free (mean 1.1 µg g^{-1} Cd, SD = 0.8); 6-month old sub-adults had moderately elevated accumulations (mean 21.4 µg g^{-1} Cd, SD = 5.8); young-adults (9 – 23 months of age) had elevated levels (mean 59.5 µg g^{-1} Cd, SD = 29.7), adults (24 months old or older) had substantially elevated levels (mean 99.4 µg g^{-1} Cd, SD = 36.6); and older adults (36 months old or older) had cadmium levels above the toxic threshold (mean 100.5 µg g^{-1} Cd; SD = 21.4). An age-dependent pattern of accumulation was not discernible among birds in the reference population.

Table 2: Age-dependent kidney-cadmium accumulations in ptarmigan in Colorado (μg g^{-1} ww)

Age	Mean	SD	n	CV
Chicks (0 to 1 month old)	1.1	0.8	3	73%
Sub-adults (6 months old)	21.4	5.8	3	27%
Yearlings (9 to 23 months old)	59.5	29.7	7	50%
Adults (24+ months old)	99.4	36.6	35	37%
Adults (36+ months old)	100.5	21.4	7	21%

When kidney-Cd levels were examined among known-age birds (banded either as chicks or as sub-adults with pigmented ninth primaries), a constant rate of accumulation was observed (Figure 2). This rate was calculated to be approximately 0.5 μg Cd/day of exposure. By projecting this rate over the lifetime of the ptarmigan, I estimated that the average ptarmigan in the minerals zone of Colorado accumulates potentially toxic levels of cadmium (= 100 μg g^{-1}) after 600 to 800 days of dietary exposure. Given the high dietary exposure rate measured in ptarmigan in the minerals-rich zone of Colorado, such an accumulation rate is reasonable and would necessitate a gut absorption rate of less than 1 %. Older birds (those with significant renal tissue damage) appear to excrete cadmium and other metals.

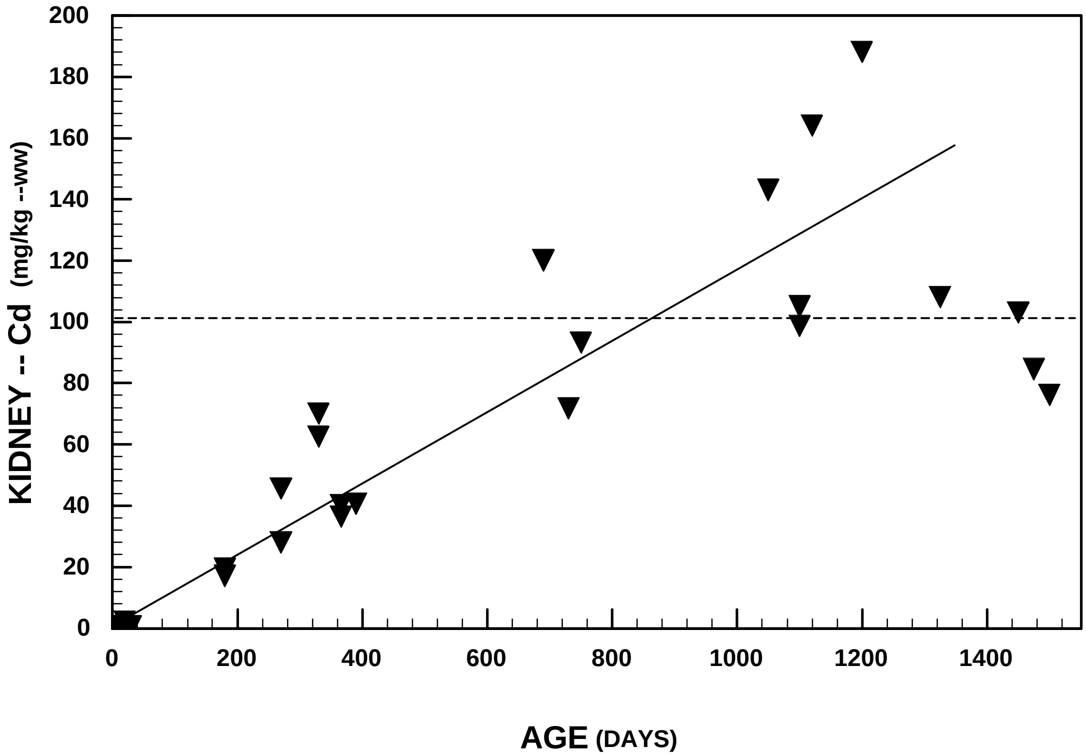

Figure 2. Kidney-cadmium accumulations in known-aged White-tailed Ptarmigan in Colorado. Toxic threshold (dashed line) from Furness 1966.

Histopathological examinations were performed on 39 ptarmigan kidneys, 12 from Alaska and 27 from Colorado. Among the Colorado birds: 9 were males, 17 were females, 1 was a chick of unknown sex, 3 were yearlings, and the remaining adults were of varying ages. Renal tubular damage (nephrosis) was observed in five females and eight males. Light-to-moderate numbers of dilated tubules lined by attenuated epithelium were observed in 4 adult females. Mononuclear interstitial inflammatory cell infiltrates were observed in five additional males. Amorphous concretions or fine granular metals deposits were observed in one female and three males. All male ptarmigan from the Colorado mineral zone had some sign of cellular damage in association with metals deposits. The single chick kidney from Colorado, all kidneys from Alaska, and all three kidneys from yearling ptarmigan from Colorado were unremarkable, showing no significant cellular damage. Most (57%) adult White-tailed Ptarmigan from the minerals zone of Colorado had some renal damage. This damage was most severe among over-wintering females, and was most common among older adults. Forty-three percent of adults and all sub-adults had unremarkable kidneys. Pancreatic tissue was unremarkable in all cases.

Chemical Content of Bone

Femur ash, calcium, phosphorus, and calcium/phosphorus ratios were lower in White-tailed Ptarmigan with kidney-Cd levels greater than 100 µg g^{-1} than in ptarmigan from Colorado or Alaska with kidney-Cd levels below this threshold (Table 3). Femurs of birds with kidney-Cd levels greater than 100 µg g^{-1} contained 8—10 % less bone ash and bone calcium than birds with lower levels. The difference in means between these two groups was not statistically significant for femur ash ($P < 0.09$) but was highly significant for femur calcium ($P < 0.01$). The calcium content in both the femur and tibiotarsus was inversely correlated with kidney-cadmium levels. The correlation was strongest in the femur.

Table 3: Femur ash salt (%) and metals (ppm) concentrations in White-tailed Ptarmigan from Alaska and two groups of ptarmigan from Colorado–one with kidney-Cd levels greater than 100 $\mu g\ g^{-1}$, the other with lower kidney-Cd levels.

Category	Ash	Ca	P	Ca/P Ratio	Cd	Cu	Zn
Colorado							
< 100	34.1	40.1	20.8	1.96	0.50	2.6	332
> 100	31.1	37.8	20.5	1.85	0.91	2.6	313
Alaska	34.3	41.1	21.1	1.94	0.23	2.7	331
Poultry*	40 – 57	40 – 57	35 – 52	3.5 – 4.0	--	--	400+

* (Puls 1988)

The calcium/phosphorus ratio was low for all ptarmigan examined. It was especially low (1.85) for ptarmigan with high kidney-Cd levels. Although, generally speaking, bone does not accumulate high levels of cadmium, femur levels were 68 % higher in the mineral zone and 82 % higher for ptarmigan with kidney-Cd levels greater than 100 $\mu g\ g^{-1}$ than for ptarmigan with non toxic kidney-Cd levels. Mean femur-zinc and copper levels were not significantly different ($P > 0.05$).

DISCUSSON

The minerals zone of Colorado contains unusually high concentrations of a number of potentially toxic trace metals; this much has been known for some time. The value of this study is that it provides evidence to support the hypothesis that at least some of these metals travel from the abiotic environment to terrestrial ecosystems by way of certain plants. The genus *Salix* appears to be the primary vector. Any herbivore consuming plants of this genus is potentially at risk of metals poisoning. We do not as yet know the extent to which past (or present, for that matter) mining operations contribute to the movement of metals from soils to ecosystems but it is likely that human activities in the ore belt region of Colorado have accelerated this process.

This study focuses on cadmium and uses the White-tailed Ptarmigan as an indicator of ecosystem health. It answers many questions about how cadmium moves through the food web and concentrates in ptarmigan renal and hepatic tissue. It documents toxicity, nephrosis, and declines in bone-calcium levels. But we do not yet know the effects of these metals on ptarmigan fitness.

It is now clear that cadmium affects ptarmigan in the Colorado ore belt, but we do not yet know the affect of cadmium on other herbivores in the region? Are elk, moose, beaver, snowshoe hare, or rodents in the area at risk? And what of the carnivores that regularly consume cadmium-contaminated herbivores. Are they at risk? And, finally, this research begs the question, what affect is cadmium having on humans in this region?

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